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# Relative abundances of the recently introduced barnacles, Megabalanus coccopoma and an unidentified species of Megabalanus, in the southeastern U.S.

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***Relative abundances of the recently introduced barnacles, Megabalanus coccopoma and an unidentified species of Megabalanus, in the southeastern U.S.***

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in Department of *Biology*

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Under the mentorship of Dr. J. Scott Harrison

ABSTRACT

*Megabalanus coccopoma* is a prominent invasive species off the coast of Georgia. Recently, among collected samples thought to be *M. coccopoma,* several individuals of an unidentified species of barnacle were found. The species has been identified as a *Megabalanus species*, but is still unidentified to the species level. Species identification is difficult due to morphological variation, inconsistent taxonomic keys, and unknown origin. In this study I developed a method to accurately distinguish *M. coccopoma* from the unidentified *Megabalanus sp.* using sequence differences in the mitochondrial Cytochrome Oxidase I (COI) gene. This study will provide an accurate estimate of the relative abundance and distribution of the unknown species and *M. coccopoma* at 7 locations off the coast of Georgia including buoys, offshore towers, and intertidal sites. The two species had different distributions. *Megabalanus coccopoma* was found at all sites and the unidentified *Megabalanus sp.* was only found at offshore sites. At the offshore sites, the two species occurred in equal abundances.

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## INTRODUCTION

An introduced species by definition is one that is not indigenous to a geographic area. If the introduced species adapts to the new environment, a potential exists for this species to out compete the native species, which can result in extinction of the native species (Lundquist *et al.,* 2003). Ecological changes that the non-native species create are a serious threat to global biodiversity (Bax *et al.,* 2003).

An introduced species must overcome several potential obstacles in order to successfully establish a population in a new area. Propagule pressure is a large factor in the successful establishment of an introduced species. Propagule pressure is the number of founding individuals introduced to a location (Lundquist *et al.,* 2003). If too few individuals colonize a new area, they may not be able to find mates or low genetic diversity may hinder population growth. Even if there is high propagule pressure, the introduced species must have enough space and hospitable environmental conditions to establish a reproductive population (Cohen *et al.,* 2014, Adams *et al.,* 2014).

Low genetic diversity due to bottleneck and founder events has traditionally been considered a major hurdle for the establishment of an introduced population (Lundquist *et al.,* 2003). However, if the propagule pressure is high, reduction of genetic diversity can be small due to minimal bottleneck and founder effects (Lundquist *et al.,* 2003, Cohen *et al.,* 2014). The rate of genetic exchange between populations can also be increased by human activities, and many introduced populations are more genetically diverse than expected

(Ellstrand *et al.,* 2000, Lundquist *et al.,* 2003). This is often due to introductions from several different source populations resulting in interpopulation hybridization in the introduced range (Lundquist *et al.,* 2003, Ellstrand *et al.,* 2000). For example, the brown anole, *Anolis sagrei*, native to the Caribbean is an introduced species in Florida. At least eight introductions of the lizard have been made in Florida from different source populations. Due to blending of genetic variation, the introduced populations have become a more genetically diverse than the native source (Kolbe *et al.,* 2004). Hybridization between several source populations in the introduced range may be one factor that enhances the establishment of the introduced species (Ellstrand *et al.,* 2000). Increased genetic diversity and hybridization between different populations of a species can enhance the ability of the introduced species to outcompete the native species and increase the potential for long-term adaptations (Lundquist *et al.,* 2003). Increased genetic diversity can also allow non-native species to quickly develop local adaptations. These adaptations may take only a few generations to arise during extreme environmental conditions with high selection pressure (Lundquist *et al.,* 2003).

An introduced species is considered invasive if it alters the economic, environmental, and ecological state within a community (Bax *et al.,* 2003). When introduced species establish themselves in a new habitat they create an overall change in the surrounding environment (Bax *et al.,* 2003). Darwin made the observation during his research that non-native species have a high tendency to outcompete and overtake the environment of the native species (Ellstrand *et al.,*

2000). If this occurs, the native species may become dominated and possibly become extinct in the area. There are several reasons why an introduced species may outcompete species native to an area, The introduced species may be more competitive due to rapid growth and reproduction. The introduced species may also have fewer limitations due to the lack of natural predators or the ability to adapt more effectively to the environment (Lundquist *et al.,* 2003). For example, a species of invasive crab, *Carcinus maenas*, has negatively affected the bivalve fisheries on the east coast of the United States, and has begun to outcompete many bird populations that consume the bivalves on the west coast of North America (Lundquist *et al.,* 2003).

Invasive species not only threaten biodiversity but also cause many negative economic and social impacts. Annually, invasive species cause damages of approximately $125 billion in the United States (Lundquist *et al.,* 2003). Industries such as fishing and tourism are highly affected by these invaders (Bax *et al.,* 2003). Also, human health can be altered due to foreign viral and bacterial pathogens brought into an environment by invasive species (Bax *et al.,* 2003). Through studies of critical life stages, ecology, genetics, and evolution of invasive species, the mechanism of how a species becomes invasive many be discovered (Lundquist *et al.,* 2003).

Many marine invertebrates have a high tendency to become invasive due to high dispersal rates and high fecundity (Cohen *et al.,* 2014). Many marine species exhibit two life stages. The first is a pelagic larval stage where the larvae move by ocean currents created by geographical and tidal forces (Adams *et al.,* 2014).

The second phase is the sessile adult phase where the individual establishes itself in an environment (Adams *et al.,* 2014). Therefore, even though some organisms have a sessile adult state, their pelagic larval state gives them the potential to disperse from the habitat where they were released (Cohen *et al.,* 2014).

The shipping industry has been a major factor in the dispersal of marine invertebrates (Miller *et al.,* 2011, Davidson *et al.,* 2008, Sylvester *et al.,* 2011). Shipping has contributed to species introductions for hundreds of years. Today, approximately 90% of the world trade is carried out through shipping (Sylvester *et al.,* 2011). As the frequency of shipping increases; a positive correlation can also be seen in the amount of species invasions (Cohen *et al.,* 2014, Davidson *et al.,* 2008). A study done in Australia, United States, and New Zealand ports discovered that a new species is introduced into the ports every 35-85 weeks (Bax *et al.,* 2003). Ships now travel faster and stay in ports longer, which allows for the survival of more species than in previous eras (Kerckhof *et al.,* 2010). Two common structures of ships that allow for the transport of marine biota are hull fouling and ballast water (Davidson *et al.,* 2008, Miller *et al.,* 2011). The hull is the exposed under water structural portion of the ship (Davidson *et al.,* 2008). This surface allows the attachment of sessile organisms such as barnacles (Cohen *et al.,* 2014, Bax *et al.,* 2003). Recently, the probability of attachment by organisms has been reduced by antifouling paints; however the paint chips often and is not effective against all species (Bax *et al.,* 2003, Yamaguchi *et al.,* 2009). The ballast water stabilizes the boat when not carrying cargo and is discharged

once the ship has reached the port (Sylvester *et al.,* 2011). The ballast water in ships can contain around 10,000 species at any given moment (Bax *et al.,* 2003). Many species cannot survive in this dirty, dark environment or die when dumped near the port (Bax *et al.,* 2003). However, some larval forms such as barnacle larvae are able to survive in these conditions and are introduced to new habitats through this mode of dispersal (Cohen *et al.,* 2014). Ballast water exchange laws (BWE) are implemented by many countries to reduce the introduction of new species into non-native ports (Miller *et al.,* 2011). The laws require ships to replace ballast water with open ocean water. These changes have reduced the amount of introductions, but introductions still occur especially during coastal travel.

Many manmade structures such as piers, docks, buoys, towers, breakwaters, jetties, and seawalls have been built to accommodate human activities in coastal areas (Lundquist *et al.,* 2003, Bulleri*.,* 2009). These structures have caused many ecological changes to the coastal habitats and promote the establishment of introduced and invasive species by providing the proper habitat (Fauvelet *et al.,* 2012). The habitats differ greatly from natural habitats, such as rocky structures, and are made of unnatural materials (Fauvelet *et al.,* 2012, Bulleri 2009). Barnacle and other invertebrate species often attach themselves to these surfaces and release larvae (Yamaguchi *et al.,* 2009). The coast of Georgia for example contains many structures such as piers, docks, buoys, and towers that provide an environment for many species to settle on and is possibly a conduit for the dispersal of introduced species.

Barnacles are a very common and successful marine invertebrate invasive species (Cohen *et al.,* 2014). Barnacles typically spend two or more weeks in the larval phase, which allows for large range dispersal of individuals (Roughgarden *et al.,* 1985). The larval stage is free floating and can easily be pulled in and later released with ballast water (Adams *et al.,* 2014). Many adult barnacles can also be found on the hulls of ships (Cohen *et al.,* 2014). Once barnacles have been introduced into a new location, the identification of species is often difficult (Cohen *et al.,* 2014). This is due to several factors including poor taxonomic information and variation in characters used to identify species (Henry *et al.,* 1986, Cohen *et al.,* 2014). If the species is an introduced species, identification is even more difficult due to the unknown geographic origin (Cohen *et al.,* 2014).

One of the many introduced species off the coast of Georgia is the barnacle *Megabalanus coccopoma*. *Megabalanus coccopoma* was described by Darwin in 1854 and has been known as many different names over time (Henry *et al.,* 1986). It is native to the eastern pacific ranging from Baja California to Peru (Crickenburger & Moran, 2013). Introduced populations of *M. coccopoma* have been discovered in Brazil, Japan, the Gulf and Atlantic coasts of the southeastern United States and most recently off the west coast of Africa (Cohen *et al.,* 2014, Yamaguchi *et al.,* 2009, Crickenburger & Moran, 2013, Newman *et al.,* 1988, Kerckhof *et al.,* 2010, Perreault 2004). *Megabalanus coccopoma* commonly attaches to recently disturbed structures located in the intertidal and sub tidal ranges. This species seems to be successful in regions with warm tropical water, and does not have a large range of thermal tolerance (Crickenburger & Moran,

2013). In the winter of 2009/2010, temperature in the southeastern United States were colder than any temperatures seen in the last 30 years. Due to this event, *M. coccopoma* populations died off from coastal sites from North Carolina to Florida but remained in offshore locations (Crickenburger & Moran, 2013). The high fecundity, rapid maturation, and aggregative settlement allowed for rapid range expansion and resettlement in subsequent years (Crickenburger & Moran , 2013). In southeastern United States, the recruitment period for *M. coccopoma* occurs from May to July and approximately 30,000 naupli are created each spawning (Crickenburger & Moran, 2013, Gilg *et al.,* 2010).

Recently, among collected samples thought to be *M. coccopoma,* several individuals of an unknown species were found. The species fit the description of the genus *Megabalanus* and looked similar to *M. coccopoma* (Fig. 1). This species is not native to the region as no *Megabalanus* barnacles are native to the southeastern United States (Henry *et al.,* 1986). It is very likely that this species has been mistaken as *M. coccopoma* in recent publications. Therefore, there are likely two invasive *Megabalanus* species in the southeastern United States, *M. coccopoma* and a second undocumented species.

There are two main objectives of the study presented here. The first objective is to develop a method using Cytochrome Oxidase 1 (COI) sequence data to distinguish an unidentified *Megabalanus* species from *M. coccopoma,* and the other ten *Megabalanus* species where sequence data is available. The second objective is to use this method to confidently distinguish the unknown species from *M. coccopoma* to estimate the relative abundance and distribution of the

*Megabalanus* species off the coast of Georgia. This will provide important ecological data on this undocumented introduced species.

*M. coccopoma* *Megabalanus sp.*

**Fig. 1.** Examples of *M. coccopoma* and unidentified *Megabalanus* specimens collected from Atlantic coast of Georgia.

QUESTIONS

1. Is the relative abundance of the *Megabalanus sp.* and *M. coccopoma* equal off the coast of Georgia?
2. What are the distribution patterns of *M. coccopoma* at sites along the coast of Georgia?

THESIS

The unknown *Megabalanus* species has most likely been present off the coast of Georgia for some time, but due to morphological similarities was identified as *M. coccopoma*. This study will provide an accurate estimate of the population size and distribution of the unknown species and *M. coccopoma* through creating an accurate distinction of the two species through genetic analysis.

METHODS

Collection sites

Specimens of *Megabalanus* were collected in Fall 2013 from seven locations; three coastal and four offshore sites off the coast of Georgia (Fig. 2). The coastal sites consisted of a public pier on Tybee Island, GA (31º59'31"N, 80º50'42"W), fishing piers at Jekyll Island, GA (31°72'71"N, 81°24'59"W), and St. Simon's Island, GA (31º08'02"N, 81º23'48"W) . The offshore sites included a buoy 20 km offshore at Gray's Reef National Marine Sanctuary (31º24'00"N, 80º52'05"W) and three old Navy Towers R2 (31º22'30"N, 80º34'01"W), R8 (31º37'59"N, 79º55'29"W), and

M2R6 (31º32'01"N, 80º14'09"W) located 50 km offshore. Once collected, the barnacles were placed and stored in 90% ethanol to preserve the specimens.

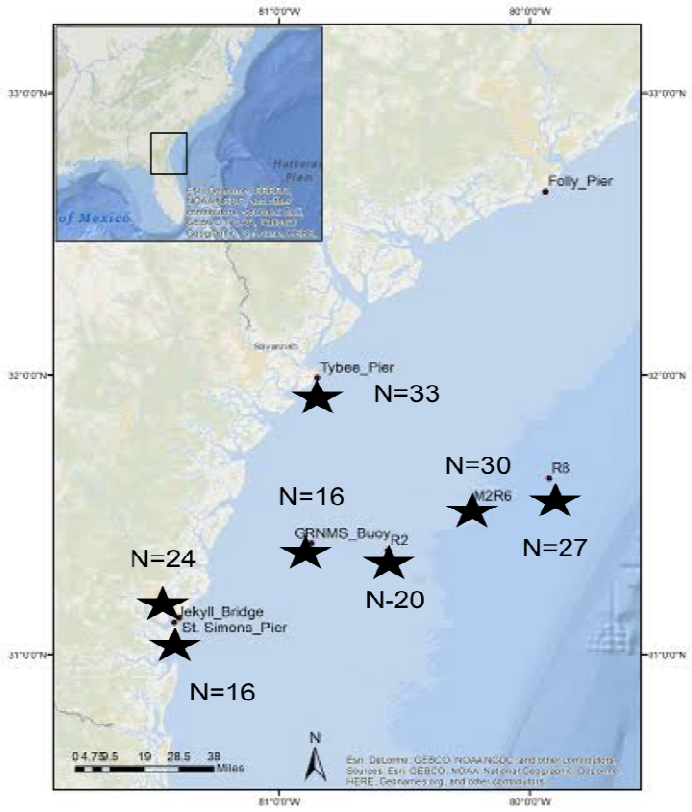


**Fig. 2.** Seven collection sites off the Georgia coast. The coastal sites include St. Simons, Jekyll Island, and Tybee Island. The offshore sites include a buoy (GRNMS) and three old navy towers (R2, M2R6, R8).

DNA Extraction and Polymerase Chain Reaction

From each site, between 16-33 specimens were used for genetic analysis. DNA was extracted and purified from each specimen following the protocol for DNeasy tissue kit QIAGEN. A portion of the mitochondrial gene Cytochrome Oxidase

I (COI) was amplified for each specimen by Polymerase Chain Reaction (PCR). I isolated the COI gene using the primers LCO1490 (5'GGTCAACAAATCATAAAGATAT TGG-3') and HCO2198 (5' TAAACTTCAGGGTGACCAAAAAATCA-3'). PCR reactions consisted of 3 µL of distilled water, 0.5 µM of forward COI primer, 0.5 µM of reverse COI primer, 0.625 units of *Taq* DNA Polymerase (Apex), 0.2 mM dNTPs, 1.5 mM $MgCl_2$, and 1 µL of DNA in a final reaction volume of 10 µL. The samples were run with the following PCR protocol: Phase 1 (94°C for 5 minutes 1 time), Phase 2 (94°C for 0.4 minutes, 55°C for 0.4 minutes, 72°C for 1 minutes repeated 35 times), and Phase 3 (72°C for 7 minutes one time).

Sequencing, Genetic Distance, and Phylogenetic Analysis

PCR products were purified using Shrimp Alkaline Phosphatase and Exonuclease I. Ten known *M. coccopoma* individuals and ten individuals thought to the unidentified species were used to obtain 700 bp of COI sequence data. Each sequencing reaction consisted of mix consisting of 3 µL of distilled water, 2 µL of 5x buffer, 0.5 µM of forward or reverse primer, 2 µL of Big Dye (Applied Biosystems), and 2.5 µL of cleaned PCR product per sample. The sequences were aligned using CLUSTALW and the uncorrected genetic distance between species was calculated using DNADIST in the SDSC Biology Workbench program. COI sequences for *M. volcano*, *M. ajax*, *M. tintinnabulum*, *M. zebra*, *M. occator*, and *M. rosa* species were obtained from GenBank. Phylogenetic relationships among species were estimated by Maximum Likelihood using the program PAML.

Restriction Enzyme Assay

Using the sequences obtained for both *M. coccopoma* and *Megabalanus sp.,* a restriction enzyme assay of COI was developed to distinguish the two species. I aligned the 700 bp sequences and chose restriction enzymes with cut sites correlating to fixed differences between species. After restriction digest of the COI PCR product, a unique banding for each species was easy to recognize on an agarose gel. The assay was composed of two enzymes Rsa1 (5´-CTˇAG-3´) and Sca1 (5´-AGTˇACT-3´). The restriction digest reaction consisted of 2 units Rsa1, 4units Sca1, 2 µL of RE buffer, 7.6 µL of di$H_2O$, and 10 µL of PCR product. The samples were incubated at 37°C for 1 hour . By observing banding patterns through gel electrophoresis, counts of each species were obtained (Fig. 3). The enzyme Rsa1 cut *M. coccopoma* at 20 bp and *Megabalanus sp.* at 300 bp, and ScaI cut only *Megabalanus sp.* at 200 bp. For *M. coccopoma*, the enzyme Rsa1 cut once at 20 bp, which yielded one 680 bp band. The *Megabalanus sp*. cut two places at 200 bp with ScaI and 300 bp with RsaI, which left bands of 100, 200, and 400 bp.

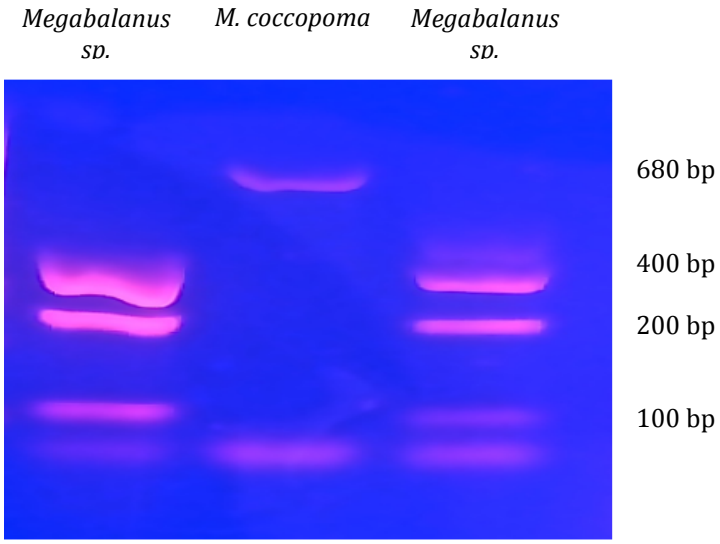


**Fig. 3.** Restriction Banding Patterns for *M. coccopoma* and *Megabalanus sp*.

RESULTS

Genetic Distance and Phylogeny

Uncorrected genetic distances between *M. coccopoma, Megabalanus sp*., *M. volcano*, *M. ajax*, *M. tintinnabulum*, *M. zebra*, *M. occator*, and *M. rosa* are provided in Table 1. The COI sequences of the unidentified *Megabalanus sp.* differed from *M. coccopoma* by 12.8%, and were 11.5-18.6% different from any other species with published COI data. The phylogeny shows that the unidentified species clearly falls within the genus *Megabalanus*. Among the *Megabalanus* species included in this study the unknown *Megabalanus* sp. is most closely related to *M. coccopoma* and *M. rosa* (Fig. 4).

**Table 1.** Genetic distance between species of *Megabalanus*.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| (1) *M. volcano* | (1) | | | | | | | |
| (2) *M. ajax* | 0.183 | (2) | | | | | | |
| (3) *M. tintinnabulum* | 0.177 | 0.156 | (3) | | | | | |
| (4) *M. zebra* | 0.162 | 0.168 | 0.072 | (4) | | | | |
| (5) *M. occator* | 0.182 | 0.173 | 0.155 | 0.143 | (5) | | | |
| (6) *M. rosa* | 0.170 | 0.193 | 0.155 | 0.156 | 0.205 | (6) | | |
| (7) *M. coccopoma* | 0.215 | 0.213 | 0.187 | 0.185 | 0.186 | 0.126 | (7) | |
| (8) Unidentified *Megabalanus* | 0.186 | 0.181 | 0.164 | 0.159 | 0.179 | 0.115 | 0.128 | (8) |

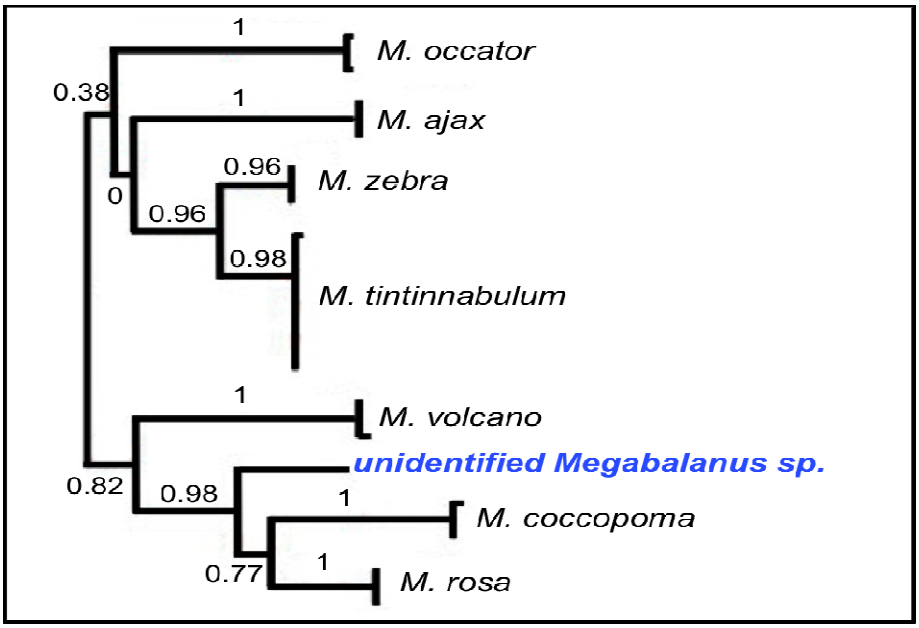


**Fig. 4.** Phylogeny of *Megabalanus* species with published COI sequence data.

Relative Abundance and Distribution

*Megabalanus sp.* was only present at the offshore tower sites, where it co-occurred with *M. coccopoma* (Fig. 5). A total of 166 barnacles were collected from 7 sites. At the coastal sites all specimens were *M. coccopoma*: St. Simons Pier (n=16), Jekyll Bridge (n=33), and Tybee Pier (n=24), Buoy (n=16). The Tower R2 site(n=20)

contained 25% *Megabalanus sp.* and 75% *M. coccopoma*. The Tower M2R6 site (n=30) contained 43% *Megabalanus sp.* and 57% *M. coccopoma.* The Tower R8 site (n=27) contained 44% *Megabalanus sp*. and 56% *M. coccopoma*. At these tower sites, the relative abundances of the two species were similar ($\chi^2$ = 2.22, df=2, p = 0.329).

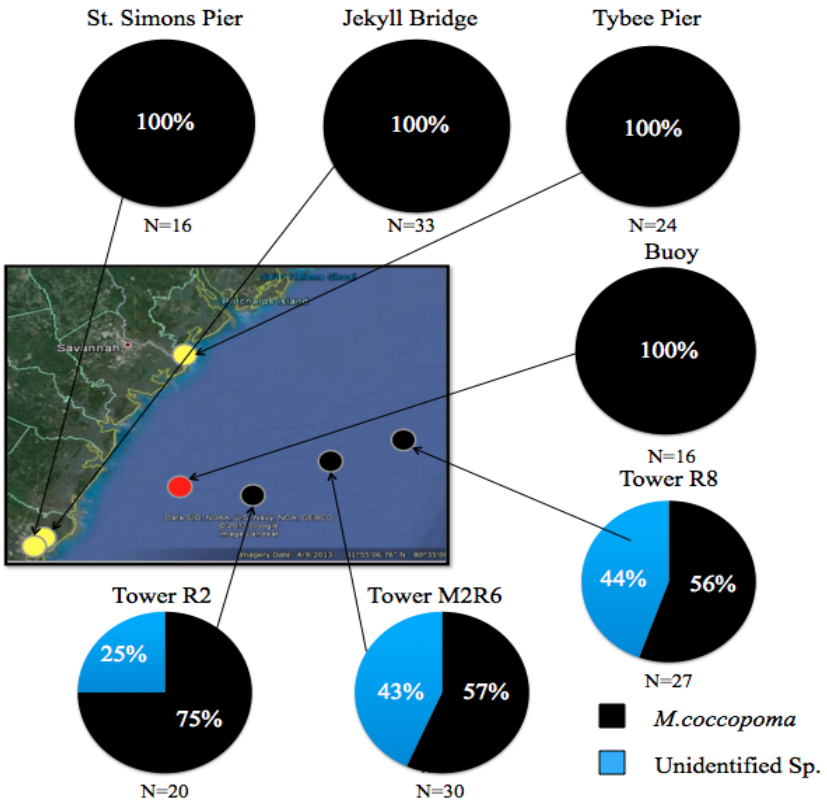


**Fig. 5.** Relative abundance of *M. coccopoma* and the unidentified *Megabalanus* sp. from three coastal (yellow), one buoy (red), and three navy towers (black). At the tower sites, *M. coccopoma* and *Megabalanus sp.* were found in approximately equal abundances ( $\chi^2$ = 2.22, df=2, p = 0.329). Only *M. coccopoma* was found at the coastal and buoy sites.

DISCUSSION

*Megabalanus coccopoma* is currently the only introduced *Megabalanus* species documented to occur off the Georgia coast (Crickenburger & Moran, 2013, Cohen *et al.,* 2014, Spinuzzi *et al.,* 2013). This study is the first documentation of a second introduced *Megabalanus* barnacle off the coast of Georgia. *Megabalanus coccopoma* and the unidentified species remain difficult to distinguish with morphological characters, but can be accurately distinguished with sequence data of the COI gene.

Barnacles in general are capable of long-range expansion due in part to a dispersing larval stage lasting greater than two weeks (Crickenburger & Moran, 2013, Spinuzzi *et al.,* 2013). This characteristic has likely played a role in *Megabalanus coccopoma's* international range expansion (Crickenburger & Moran, 2013, Cohen *et al.,* 2014). The new introduced *Megabalanus sp.* may have a similar ability to be transported to new environments via vessels such as through the shipping industry (Davidson *et al.,* 2008, Kerckhof *et al.,* 2010). Even though a species may be able to transport individuals to a new area, they must be able to survive in the ecological conditions of that area. The unidentified *Megabalanus sp.* and *Megabalanus coccopoma* appear to differ in the conditions in which they can survive in coastal Georgia.

The genus *Megabalanus* is notorious for exhibiting phenotypic characteristics difficult to distinguish (Cohen *et al.,* 2014) . Darwin spent years of his research discovering morphological characteristics between species of *Megabalanus* barnacles (Mannouris *et al.,* 2011, Newman *et al.,* 1987). As seen in Fig. 1, *M.*

*coccopoma* and *Megabalanus sp.* have very similar morphological characteristics. Although some taxonomic keys exist, the morphological differences listed are not sufficient to distinguish between the two species consistently (Cohen *et al.,* 2014).

In my study, I implemented COI barcoding to compare sequences with other species of the same genus. Using sequences posted in the GenBank database, I was able show that the sequence for the unknown *Megabalanus sp*. exhibited a large sequence divergence compared to all published sequences (Table 1). Sequence divergence for COI is typically under 3% among individuals of the same *Megabalanus* species (Cohen *et al.,* 2014). The sequence divergence reported here is clearly outside this range. Using phylogeny, the sequence of the unknown species confidently grouped within the genus *Megabalanus* (Fig. 4). DNA barcoding in recent years has become a useful to tool to distinguish between cryptic species. Now many taxonomists use genetic data as additional traits to support their results (Hebert *et al.,* 2005). In one study, the species of neotropical skipper butterfly, *Astraptes fulgerator,* was considered to be one species using morphological data only. Following DNA barcoding of COI, ten different species were identified (Hebert *et al.,* 2004). DNA barcoding can greatly aids in the identification of species through improved accuracy and speed (Hebert *et al.,* 2004, Hebert *et al.,* 2005).

The artificial structures present on many coastlines create a habitat for many introduced marine species (Astudillo *et al.,* 2009, Bulleri 2009, Fauvelot *et al.,* 2012). Barnacles often attach to structures such as bridges, buoys, and towers and quickly reproduce (Cohen *et al.,* 2014, Crickenburger & Moran, 2013). All of the sampling sites in my study were artificial structures, which have clearly facilitated the

introduction of *M. coccopoma* to the area. *M. coccopoma* was found at both coastal and offshore sites, while the unidentified *Megabalanus* species was only found at the offshore towers. On the towers, the abundance of the unidentified barnacle was roughly equal to *M .coccopoma* (Fig. 5). These structures often serve as a conduit for introduced species to establish themselves in non-native territory (Astudillo *et al.,* 2009, Bulleri 2009, Fauvelot *et al.,* 2012, Cohen *et al.,* 2014).

In addition to the genetic differences between *M. coccopoma* and the unidentified *Megabalanus* sp., ecological differences also appear to distinguish the two species. The differences in the distribution of the two species may be a result of different abilities of the two species to tolerate this environmental variability in coastal and offshore sites. Crickenburger & Moran (2013) showed that *M. coccopoma* experienced a die back on the southeastern coast of the United States during an especially cold winter. *M. coccopoma* does not have a wide range of tolerance of temperature and salinity which is typical of a tropical species (Crickenburger & Moran, 2013, Glig *et al.,* 2010). Different levels of temperature and salinity can greatly effect recruitment and larval development (Thiyagarahan *et al.,* 2002). *Megabalanus coccopoma* has also been shown to have decreased survival in waters of high salinity (Gilg *et al.,* 2010). The distribution observed here for the unidentified *Megabalanus sp*. suggests that this species is even less tolerant of salinity and temperature fluctuations than *M. coccopoma*. The *Megabalanus sp.* was found only at tower sites and increased in abundance as the distance from the shore increased. Off the coast of Georgia, the gulf stream from the tropics passes several kilometers offshore (Fig. 7) (Lee & Brooks, 2010). The offshore structures used for

sampling are located at the edge of this current. The offshore sites therefore have less fluctuation in temperature and salinity due to their position in tropical waters (Lee & Brooks, 2010). The coastal sites are not as stable and have variable salinity and temperatures through the seasons (Fig. 6). It is possible that the towers provide a better environment for the less tolerant introduced *Megabalanus* species.

To my knowledge, no other documentation of the *Megabalanus sp.* has been made. Cohen *et al.* (2014) also found a second barnacle species collected with *M. coccopoma* samples in Florida. The barnacle was also found in tropical waters in Pensacola, Florida on the Gulf coast and Fort Piece, Florida on the Atlantic coast (Cohen *et al.,* 2014). No COI sequence data is available for these samples so comparisons could not be made to determine if it is the same species observed in this study.

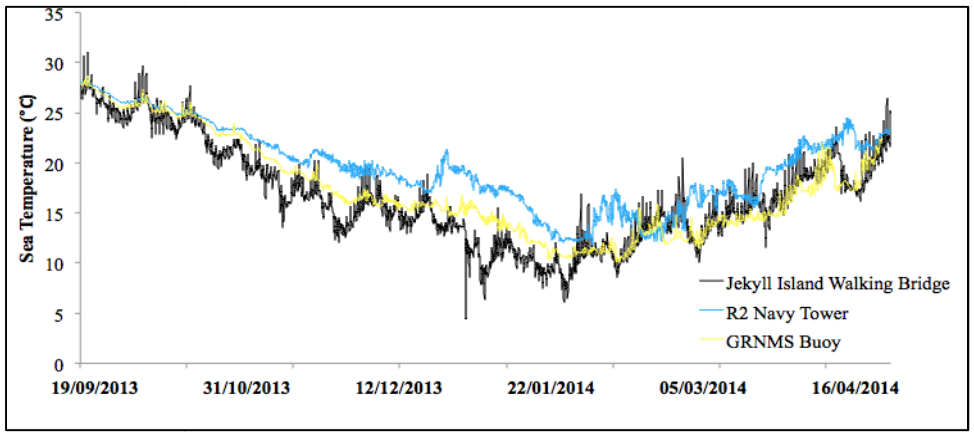


**Fig. 6.** Seasonal fluctuations in water temperature for experimental sites (Reigel, A.M. unpublished data)

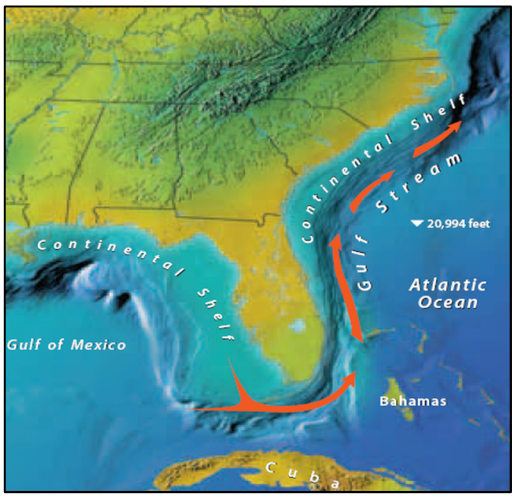


**Fig. 7.** Gulf Stream current in the Gulf of Mexico and Atlantic Ocean. (https://www.roffs.com/2014/04/seasonal-fishing-forecast-northeast-florida-northeast-canyons-looking-good-much-can-happen/)

In conclusion, I have identified an unknown introduced *Megabalanus* species off the coast of Georgia. This species may have been mistaken to be *M. coccopoma* in several published studies due to similarities in phenotypic characteristics. I have not been able to identify this *Megabalanus* species with taxonomic keys and morphological characteristics. I created a way to distinguish this species from *M. coccopoma* specimens that is relatively quick and easy. Accurate identification tools are particularly important in the context of invasive species, as several species of *Megabalanus* are expanding their range (Crickenburger & Moran, 2013). From the distribution data, I concluded that the unidentified *Megabalanus sp.* may be originate from a tropical region due to its existence only on the offshore towers located in warmer less variable waters.

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